

Synthetic gene drive: between continuity and novelty

Crucial differences between gene drive and genetically modified organisms require an adapted risk assessment for their use

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CRISPR/Cas accelerates the development of synthetic gene drive organisms to quickly spread a genetic modification among the target species. Both in academia and politics, the use of CRISPR/Cas gene drive to potentially control disease vectors, plant pests or invasive alien species is controversial, as exemplified by the last Conference of the Parties of the United Nations Convention on Biodiversity (CBD) and the most recent meeting of its scientific expert group on synthetic biology (Ad Hoc Technical Expert Group/AHTEG). While some argue that current risk assessment frameworks can accommodate synthetic gene drives, others call for a moratorium owing to gene drives' potentially detrimental impact on wildlife. In essence, the question is whether we have sufficient experience and knowledge to handle this technology safely. Experience and knowledge in turn depend on the degree of continuity and novelty of synthetic gene drive organisms (GDO), compared to existing genetically modified organisms (GMO). While gene drives exist in nature, we find that GDO differ from the currently released GMO on five levels. A clear understanding and analysis of these differences is crucial for any risk assessment regime and a socially acceptable and ethical evaluation that is vital for the application of this technology.

From nature to synthetic biology

Gene drive is a natural phenomenon by which a genetic element is transferred to more than 50% of the offspring of a sexually

reproducing organism: natural gene drives are selfish genetic elements that sidestep the rules of Mendelian inheritance, resulting in a so-called super-Mendelian inheritance. In 2003, Austin Burt proposed to use this natural phenomenon and synthetically engineer gene drives using specific enzymes called "homing endonucleases." The discovery of the natural bacterial defence system CRISPR/Cas and its application as a highly specific nuclease eventually boosted the development of synthetic gene drives to circumvent Mendelian inheritance of both the gene drive elements itself and any payload gene(s). Synthetic gene drives allow a wide range of possible applications. *Modification drives* can introduce a new trait to a target population or species, and *suppression drives* aim for population control by eradicating target populations or species [1]. The latter can for instance change the sex ratio destroying the X chromosome with the help of a gene drive mechanism called X-shredder.

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recent developments in genetic engineering, since synthetic gene drives are essentially GMO. In parallel, GDO are also conceptually and biologically novel owing to five key features, which affect the application strategy, commercialization, spread of the modification, technical realization and ecosystems.

From indirect protection to direct action

Various GM crops and, in rare cases, GM insects have been released commercially or experimentally into the environment during the past few decades. Few crops—maize, cotton, soya beans and oilseed rape—and traits, notably herbicide and insect resistance, dominate these applications, and regulation has been tailored accordingly to assess the risks to human health and the environment. Compared to current GM crops, the goals for GDO are substantially different. While the main GMO strategies aim to protect crops *from* a stressor such as insect pests or weeds by transferring genes for pest or herbicide resistance, many GDO are expected to work directly *against* a stressor: to suppress a disease vector or a pest species. This changes the ecological burden as species and habitats are affected beyond the agroecosystem. This strategy change has already been used on a much smaller scale to control local populations of insects using sterile insect technology (see Box 1). Gene drive represents a much more powerful concept that may affect a wider spectrum of organisms with higher spatial implications.

Common goods

The marketing strategy of current GM crops provides mainly advantages for producers but has often little benefit for consumers. It also restricts access to the benefits by patenting the technology and licensing the seeds, which must be newly bought by farmers each growing season. GMO can also be common goods, but such products are very rare owing to patent rights and the above-mentioned marketing strategy.

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In contrast, some GDO may well create a common good: for instance, disease control. The target malaria project is a prominent example: this non-profit consortium aims to control malaria-carrying mosquitoes using a suppression drive. It could theoretically eradicate a whole mosquito species and thereby potentially achieve a partial reduction of the global malaria burden—the first field releases are expected to occur in the following decade [2]. At the same time, public goods have to be evaluated against public burden that might arise from their ecological and socio-economic impacts for present and future generations.

Outcrossing and spread of transgenes

GM crops require extensive tests in the laboratory, greenhouse and field to obtain a marketing licence. Moreover, inheritance of the transgenes is usually undesirable owing to marketing and patenting strategies, and GM seeds are often marketed with hybrid vigour. This again changes for GDO as its functionality is based on the super-Mendelian inheritance of the genetic modification to spread in the environment. Thus, while the transfer of a transgene to wild relatives is considered a hazard in GM crops, inheritance and spread of the transgene is a required prerequisite for GDO [3]. Moreover, the interactions between a gene drive and its target populations are less predictable in

GDO than in GMO. A transgene that is outcrossed into closely related species from a classical GMO has a high chance of becoming lost through genetic drift if the allele does not provide a selective advantage. In contrast, a gene drive allele has a much higher chance of becoming established in the population, even if the payload gene may be negatively selected against under normal conditions.

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Once the gene drive allele leaves a contained environment, the level of genetic diversity in the recipient organism may also start to affect the spread of the transgene. Crops—GM or not—are bred to behave uniformly, which comes at the price of reduced genetic diversity. Wild populations, which are most likely targeted by GDO, are genetically much more diverse. Thus, the interactions between transgene and genetic background become more complex and less predictable. Unsuccessful suppression gene drives could, in addition, reduce the genetic diversity of the target species and affect the fitness of a population in ways that are hard to predict. Generally, the level of genetic diversity greatly differs among species and must therefore be carefully considered in creating and assessing GDO.

The lab in the field

As the CRISPR/Cas toolbox is inherited by GDO, the laboratory moves into the environment. Although CRISPR/Cas gene drives are constructed in the laboratory, the drive is designed to genetically modify organisms in the wild [3]. In fact, gene drives imply a shift from the release of a finished and tested product to the release of an adjustable tool for genetic modification that is released into ecosystems.

Genetic modification using gene drive affects the germline in every generation of the target organisms. The highly complex drive construct can therefore cross into wild-life in ways that are rarely characterized to

the same extent as the risk of outcrossing from classical GMO. Thus, non-intended effects, such as resistance or off-target effects, are difficult to predict and characterize before the release into the environment, particularly in genetically diverse wild populations. This also creates great challenges for any monitoring of GDO. Risk assessment of classical GMO uses a stepwise approach successively under laboratory, greenhouse and field conditions. This is not possible for GDO, at least to the point when the GDO is tested in the field, since releasing even some individuals can be considered a full release. However, a stepwise testing would be especially important for this technology given its many uncertainties. There are additional legal concerns: the authorization of a GMO release is generally time restricted to allow a feedback from monitoring and withdrawal if the organism creates environmental risks. The transgenic modification of the germline by gene drive is, by definition, not time restricted and can therefore not be withdrawn, which conflicts with the EU directive on the deliberate release of GMO into the environment.

Modifying wildlife

Probably, the most important novel aspect of synthetic gene drives is that it can be applied to wildlife, even if it may not function in all species. In fact, most gene drive-based applications that are currently being developed or proposed target invasive alien species or insect vectors for human and animal diseases. The transition from modifying cultivated species such as crop plants to modifying wild species has major consequences on semi-natural and natural ecosystems. Upon release into the environment, the genetic modification will likely invade and affect other habitats and ecosystems beyond the area of release.

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The same would hold true for gene drive applications in agriculture. After the release of a gene drive to control a “pest” insect, the genetic modifications can also enter other ecosystems, where the insect is no longer

categorized as a pest. Additionally, other strategies related to human health or conservation biology aim to affect natural habitats. To eradicate invasive rodents from islands by gene drive, GMO or, more specifically in this case, GDO must be released into natural ecosystems. Risk assessors as well as existing frameworks will be heavily challenged by this change in the spectrum of organisms and environments.

From continuity and novelty to practical consequences

Although GDO are consistent with classical GMO, we identified five novel features. We use the term GDO to address effects at the level of the organism rather than to establish a new legal category of genetically modified organisms. In biology, it is rarely possible to define clear categories, but a sufficient understanding of the novel characteristics of GDO, which are at least quantitatively new, is necessary to properly evaluate the impact of GDO on the environment. Importantly, GDO target wild species in semi-natural and natural habitats. Compared to classical GMO, the assessment of their effects therefore requires a stronger focus on the complex interactions between the modified organisms and the ecosystem. Furthermore, the impact of CRISPR/Cas gene drive systems, which have been developed only recently, is not fully understood yet—even under controlled laboratory conditions. The technical hurdles, including resistance to CRISPR/Cas gene drive mechanisms, will possibly be overcome as advanced strategies are designed, such as multiplexing of targeting sites.

As CRISPR/Cas is nearly universally applicable, GDO can, at least theoretically, be realized in most animal and plant species if the technical and/or biological constraints for plants are solved in the future [4]. Considering these prospects, GDO have a realistic potential to generate global effects at the species level. Proposed gene drive applications are broad and might affect many fields, including human health, agriculture and conservation biology [5]. However, since gene drives alter populations or species, the potential consequences on wildlife have been discussed as well [6–8]. We strongly argue that prior to the release of GDO into the environment, a wise risk assessment strategy is needed.

The intermediate position of GDO between continuity and novelty leads back

to the question whether we have gathered enough experience and knowledge to handle this technology in a safe way. In other words, do we have a suitable risk assessment regime and legislation in place or do we need a new framework? Consistent with our findings, we come to a mixed conclusion. We reason that the mere risk assessment of GDO can, in principal, be performed under the current risk assessment schemes for GMO. However, guidelines and methodologies need to be amended and operational prior to a first risk assessment, giving justice to the potent novel features of GDO described above. Several key issues in this respect need to be solved.

The safe handling of GDO in contained use needs special attention, since even a small unintended release can already lead to an extensive spread of the gene drive. The concept of biological security levels for research facilities is currently primary tailored to pathogens. For the safe handling of GDO, some measurements would be redundant, whereas others are missing [9,10]. A conceptual adaptation of these security levels to GDO, with an additional focus on potential environmental hazards, is therefore needed.

As described above, the stepwise approach of risk assessment for GMO cannot be performed as it includes field testing, which requires the release of GDO to the environment. For global gene drives, this step already represents the release itself, contradicting the intended procedure. Spatially or temporally limited gene drives are being discussed in this context, but those are lacking proof of concept. Information based on modelling and scenarios prior to release may become more important for the stepwise approach.

Finally, the assessment of long-term ecological effects, which may potentially lead to population or species extinction is, to say at least, challenging. Compared to current GM crops, the environmental risk assessment of GDO will need substantially more information and baseline data on the receiving environment and its ecosystems. Moreover, data on the genetic variability, ecology and the specific roles in ecosystems are incomplete or lacking for most wild species, including those arthropods which are likely to be targeted by gene drives. Even when data are available, appropriate effect thresholds will have to be defined and

Box 1: Comparison of transgenic sterile insect techniques and gene drive

The sterile insect technique (SIT) was developed before GMO or synthetic gene drives and has been successful in suppressing the population of some insect species. Male insects are bred, sterilized and released into natural habitats to mate with their wild relatives. Sterility prevents successful reproduction, which leads to a decline in the population numbers. More recent strategies using genetically modified sterile insects (GM-SIT) have been developed to increase efficiency, because the conventional technique has several conceptual and technical limitations. Additionally, SIT is expensive because many insects must be released repeatedly for this technique to be effective. There are important differences between gene drive, which has a self-propelling mechanism, and SIT, which also uses GM: SIT does not intend to spread transgenes in wild animal populations; modified animals are not meant to survive; SIT is generally applied locally; and GM insects typically disappear quickly after release. Thus, in contrast to GDO, SIT is time-limited. To describe gene drive as the next step from GM-SIT is an oversimplification, because gene drive represents a novel and much more powerful strategy.

agreed on. Thus, in the area of ecological impact assessment, estimating risks will become highly complex and multi-layered, especially for global gene drives.

A technology assessment approach to complement risk assessment

The novel features of GDO also lead to challenges that are not covered by current legislation. If we consider the application to modify wildlife, where scientific experience is in its infancy, and the shift in strategies that may lead to common goods, it becomes clear that mere risk assessment comes to its limit. To agree on thresholds, necessities, acceptable uncertainties and common goals, a wider societal perspective is needed. We therefore suggest a technology assessment approach that goes beyond mere risk assessment and that is generally not foreseen in legislations. On a basic level, this approach could discuss the appropriateness of the technique in comparison with other means to achieve the goal. On a wider scope, it could incorporate a certain appraisal of social, economic and cultural impacts that are not

part of the pure risk assessment. Technology assessment has developed instruments for feedback loops to society that could prove adequate given the potential impact of GDO to go global and trigger huge effects on biological diversity and the socio-economic and cultural use of biodiversity. Thus, to date, the most suitable place to discuss these potential adaptations of legislation is, where the discussion has already started, at the CBD level. Until then, scientists and regulators working on gene drive technology should take adequate precautionary measures.

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Conflict of interest

The authors declare that they have no conflict of interest.

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